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Hematology, micronuclei and nuclear abnormalities in fishes from São Francisco river, Minas Gerais state, Brazil

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ABSTRACT. This study aimed to determine the variables leukocytary, erythrocytary, frequency of micronuclei and nuclear abnormalities in peripheral erythrocytes of *Prochilodus argenteus, Pimelodus maculatus and Myleus micans* from São Francisco river basin, Minas Gerais State, during the summer and winter. Thrombocytes, hematocrit and leukocyte of *P.argenteus* series, was influenced by climatic conditions. In *P. maculatus*, not were significant differences for all leukocytes and thrombocytes. In *M. micans*, values are unprecedented in the literature, with MCV and absolute number of leukocytes found superior to other species of the family Characidae. The frequency of micronuclei and nuclear abnormalities in erythrocytes, the three species not showed seasonal differences between the sites. However, the highest values were found in summer. Furthermore, we observed a positive correlation between the increase in the percentage of micronuclei with nuclear abnormalities

Keywords: Hematological, fish, micronuclei, nuclear abnormalities, São Francisco.

RESUMO. Hematologia, micronúcleos e anomalias nucleares em peixes do rio São Francisco, Estado de Minas Gerais, Brasil. O presente estudo teve como objetivo, determinar as variáveis leucocitárias, eritrocíticas, freqüência de micronúcleos e anomalias nucleares nos eritrócitos do sangue periférico de *Prochilodus argenteus, Pimelodus maculatus* e *Myleus micans* de um trecho da bacia do rio São Francisco, Minas Gerais, no verão e inverno. Trombóctios, hematócrito e a serie leucocítária de *P.argenteus*, foi influenciado pela sazonalidade. Em *P. maculatus,* não ocorreram diferenças significativas para todos os leucócitos e trombócitos. Em *M. micans,* os valores são inéditos na literatura, apresentando VCM e número absoluto de leucócitos superior ao encontrados para outras espécies da família Characidae. A freqüência de micronúcleos e de anomalias nucleares nos eritrócitos, nas três espécies não apresentou diferenças sazonais e entre os pontos amostrais. No entanto, os valores mais altos foram encontrados no verão. Além disso, foi possível observar correlação positiva entre o aumento da porcentagem de anomalias nucleares com micronúcleos.

Palavras-chave: Hematologia, peixe, micronúcleo, anomalias nucleares, São Francisco.

Introduction

The hydrographic basin of São Francisco river occupies 631,133 km2, which represent 7.4% of Brazilian territory (PAIVA, 1982) with wide and diversified ichthyofauna. Godinho (1993), Britski et al. (1988), Sato and Godinho (1999), Alves and Pompeu (2001) reported 184 species from the fish orders Clupeiformes, Characiformes, Siluriformes, Gymnotiformes, Synbranchiformes, Cyprinodontifor mes, Perciformes characterized as endemic species. The ecophysiological parameters of these fish are practically unknown, and eventually this characterizes a limitation for assessments of environmental quality through biomarkers and culture in captivity.

The application of hematology in animal research is well accepted and considered a routine procedure in diagnostic methods. In addition, knowledge of biochemical parameters is important to understanding ecological interactions and the relationship between endogenous and exogenous factors and may thus serve as an indicator of health and effects of pollutants. However, the use of these parameters is limited by the lack of knowledge of normal values to comparisons, thus the ichthyohematology is indispensable (RANZANI-PAIVA; SILVA-SOUZA, 2004).

The presence of micronuclei in peripheral blood erythrocytes has been used as a biomarker of genotoxicity caused by pollutants since 70's (SCHMIDT, 1975). One of the advantages for their use is that 97% of fish blood cells are red blood cells, and only 3% are white blood cells, so this represents a high homogeneity (MITCHELMORE; CHIPMAN, 1998).

Micronuclei are results from acentric chromosome fragments or chromosomes that delay, in relation to others in their migration to the poles of the cell in anaphase (AL-SABT; METCALFE, 1995; HEDDLE, 1973; SCHMIDT, 1975). According Heddle et al. (1991) and Al-Sabti and Metcalfe (1995), micronuclei can also be formed by apoptosis, inactivation of the spindle formation and chromosome damage beyond action of physical agents. Other abnormalities in the nuclei of red blood cells have been mentioned as biomarkers of cyto and genotoxicity and thus used in a complementar analysis of the frequency of micronuclei. Studies by Ayllon and Garcia-Vasquez (2000) and Kirschbaum et al. (2009) showed this correlation indicating that nuclear abnormalities could be primary responses, i.e., prior the formation of micronuclei.

Species respond differently to environmental stimuli and in any specimens have a basal rate of formation of abnormal cells. Therefore, this knowledge may aid to choice of which specie respond significantly indicating the presence of pollutants through biomarkers.

In this context, this study aimed to determine the baseline frequency of micronuclei and nuclear abnormalities in erythrocytes of peripheral blood and describe the types of leukocytes, and erythrocytic characteristics of three tropical fish with importance for aquaculture and biomonitoring: *Prochilodus argenteus* Agassiz, 1829, *Pimelodus maculatus* Lacepède, 1803 and *Myleus micans* Lütken, 1875, of the rivers, Paracatu and San Francisco, at Minas Gerais State, Brazil, in two periods: summer (January) and winter (June).

Material and methods

We used three species: *P. argenteus* (n = 13, winter / n = 11, summer) with weight 943.0 + 359.3 g and length 37.0 + 2.0; *P. maculatus* (n = 21, winter / n = 11, summer) with weight and length 211.0 + 39.0 g and 23.5 + 0.5 cm respectively, and *M. micans* (n = 10, winter) with 430.0 + 0.14 g and 26.9 + 1.9 cm. The fish were collected in the São Francisco river (Três Marias) and Paracatu river (Brasilândia de Minas), during the months of January and June.

After the capture, they were placed in containers with aerated river water and taken to the laboratory. After acclimation to reduce the stress of capture and transport, fish were anesthetized with benzocaine (3%) and we performed biometrics and blood sampling by caudal puncture with the aid of needles and syringes heparinized. With blood samples were determined: total number of erythrocytes (Er) in Newbauer chamber, hematocrit (Ht%) by the method of microhematocrit (GOLDENFARB et al., 1971) and hemoglobin (Hb) by the cianometahemoglobin method (COLLIER, 1944). We calculated the mean corpuscular volume (MCV = Ht / Er x 1000) expressed in fentoliter (fL) and mean corpuscular hemoglobin concentration (MCHC = Hb / Ht x 1000) expressed in grams per deciliter (g dL^{-1}).

Slides were stained with May-Grunwald-Giensa (ROSENFELD, 1947) for differential count (percentage) and total leukocytes and thrombocytes, according to the indirect method adopted by Hrubec and Smith (1998) for determining the absolute number of each leukocyte.

The frequency of erythroblasts, micronuclei and nuclear abnormalities were estimated by counting 2000 cells in extensions. The nuclear abnormalities were grouped together irrespective of form in a single class, in accordance with the method used by Kirschbaum et al. (2009).

The Shapiro-Wilk Test for normality was performed. The results were submitted to the Student test "t" and Pearson correlation for micronuclei, nuclear abnormalities and erythrocytic variables (ZAR, 1999) was done.

Results

The erythrocyte variables of species are in Table 1. In *P. argenteus* no statistical difference was found for any of the variables between the fish of the rivers São Francisco and Paracatu. In the São Francisco river the Ht value was higher during the summer. There was no statistical difference for the other indices.

The absolute number of each type of white blood cell, total leukocytes and thrombocytes are in Tables 2 and 3. In *P. argenteus* from São Francisco river, the absolute value of neutrophils and eosinophils in summer were higher than in fish from Paracatu river. Regarding seasonality, the total number of leukocytes and absolute neutrophils, monocytes, eosinophils and basophils were statistically different between the summer and winter. Significant difference statistical in the number of thrombocytes between rivers and seasons was observed.

Pimelodus maculatus from São Francisco river had no significant variation regarding seasonality of Ht, MCV, MCHC and Hb. The Er was significant difference statistical between the winter and summer. The leukocytes did not present significant difference statistical in the total number of leukocytes, absolute number of leukocytes and thrombocytes.

The frequency of micronuclei and nuclear abnormalities in erythrocytes are presented in Table 4. Significant difference statistical was not found in individuals of *P. argenteus* collected in the rivers São Francisco and Paracatu. The seasonality did not affect significantly the frequency of nuclear abnormalities, micronuclei and erythroblasts. In *P. maculates* we did not observe changes in relation to seasonality. The values observed for *M. micans* was similar to *P. argenteus* and *P. maculatus*.

Table 1. Erythrocytic variables of *P. argenteus, P. maculatus* and *M. micans* of the São Francisco and Paracatu rivers, Minas Gerais State, where (*) indicates statistical difference ($p < 0.05$).

Summer							
River	Species	Erythroblasts (2000 cells)	Hematocrit (%)	Erythrocytes (10^6 uL)	Hemoglobin (g dL^{-1})	MCV (fL)	MCHC (g dL^{-1})
S. Francisco	*P. argenteus*	2.7 ± 2.2	42.7 ± 3.0**	217.7 ± 38.3	8.9 ± 1.2	212.0 ± 38.0	21.0 ± 2.3
Paracatu	*P. argenteus*	1.6 ± 1.4	38.4 ± 6.8	218.9 ± 57.7	8.5 ± 1.6	212.0 ± 188.3	22.3 ± 3.2
S. Francisco	*P. maculatus*	2.1 ± 2.0	45.0 ± 5.0	257.8 ± 53.62*	9.1 ± 1.2	205.6 ± 57.4	20.2 ± 2.5
Winter							
River	Species	Erythroblasts (2000 cells)	Hematocrit (%)	Erythrocytes (10^6 uL)	Hemoglobin (g dL^{-1})	MCV (fL)	MCHC (g dL^{-1})
S. Francisco	*M. micans*	2.6 ± 9.3	32.9 ± 7.4	126.1 ± 36.5	7.1 ± 1.7	279.0 ± 102.4	21.5 ± 2.1
S. Francisco	*P. argenteus*	3.8 ± 4.5	35.4 ± 5.4	198.6 ± 42.0	16.1 ± 2.1	186.8± 5.4	45.7 ± 28.5
S. Francisco	*P. maculatus*	2.1 ± 2.0	40.0 ± 2.0	200.8 ± 23.6	9.6 ± 1.2*	206.0 ± 57.4	20.3 ± 2.3

* = difference from the same specimen, ** = sazonal difference.

Table 2. Absolute number of lymphocytes, neutrophils, monocytes, eosinophils and basophils of *P. argenteus, P. maculatus* and *M. micans* from São Francisco and Paracatu rivers, Minas Gerais State, where (*) indicates statistical difference ($p < 0.05$).

Summer						
River	Species	Lymphocytes (μL)	Neutrophils (μL)	Monocytes (μL)	Eosinophils (μL)	Basophils (μL)
S. Francisco	*P. argenteus*	11234.7 ± 7056.2	17767.6 ± 7771.8*,**	1783.1 ± 797.8*,**	1081.8 ± 707.3*,**	2073.6 ± 2013.7*,**
Paracatu	*P. argenteus*	6638.6 ± 7268.7	10451 ± 6679.0	1948.4 ± 1193.0	378.7 ± 436.1	2083.2 ± 3418.0
S. Francisco	*P. maculatus*	8558.1 ± 3468.32	19961.4 ± 13329.0	1846.0 ± 691.9	7.7 ± 2.5	43.6 ± 63.83
Winter						
River	Species	Lymphocytes (μL)	Neutrophils (μL	Monocytes (μL)	Eosinophils (μL)	Basophils (μL)
S. Francisco	*M. micans*	10192.7 ± 7056.2	929.7 ± 1332.5	1163.5 ± 1113.0	1565.8 ± 1354.2	302.6 ± 754.7
S. Francisco	*P. argenteus*	7610.3 ± 4184.1	4179.3 ± 3259.6	447.7 ± 286.7	313.3 ± 540.1	323.3 ± 519.7
S. Francisco	*P. maculatus*	13214.5 ± 11648.4	1625.1 ± 964.4	10807.4 ± 10130.3	669.1 ± 920.5	34.52 ± 56.78

* = difference from the same specimen, ** = sazonal difference.

Table 3. Total number of leukocytes and thrombocytes of *P. argenteus, P. maculatus* and *M. micans* from São Francisco and Paracatu rivers, Minas Gerais State, where (*) indicates statistical difference ($p < 0.05$).

Summer			
River	Species	Leukocytes (μL)	Thrombocytes (μL)
S. Francisco	*P. argenteus*	34012 ± 14661**	17767.6 ± 7771.8*,**
S. Francisco	*P. maculatus*	30584 ± 15465	19961.4 ± 13329.0
Winter			
S. Francisco	*P. argenteus*	18175 ± 8530	5440 ± 2484
S. Francisco	*P. maculatus*	13214 ± 11648	14571 ± 11314
S. Francisco	*M. micans*	14742 ± 9340	12948 ± 7192
Summer			
S. Francisco	*P. argenteus*	20412 ± 14661	11165 ± 10119
Paracatu	*P. argenteus*	21610 ± 15109	13735 ± 22001

Table 4. Frequency of micronuclei and nuclear abnormalities in peripheral blood erythrocytes of *P. argenteus*, *P. maculatus* and *M. micans* deriving from the rivers São Francisco and Paracatu, Minas Gerais State.

Summer			
River	Species	Micronuclei (2000 cells)	Nuclear Abnormalities (2000 cells)
S. Francisco	*P. argenteus*	0.7 ± 0.4	3.8 ± 1.5
Paracatu	*P. argenteus*	0.2 ± 0.1	1.0 ± 0.4
S. Francisco	*P. maculatus*	0.1 ± 0.04	0.6 ± 0.2
Winter			
S. Francisco	*M. micans*	0.7 ± 0.3	3.8 ± 1.5
S. Francisco	*P. argenteus*	0.2 ± 0.1	1.0 ± 0.4
S. Francisco	*P. maculatus*	0.1 ± 0.04	0.6 ± 0.2

Furthermore, we did not verify correlation between micronuclei, nuclear abnormalities, Er, VCM and erythroblasts in *P. argenteus* from São Francisco river during both periods. In the specimens from the Paracatu river we observed positive and significant correlation between MCV increase and erythroblasts. In *P. maculatus* there was no significant correlation for any of the erythrocyte variables with micronuclei and nuclear abnormalities in the summer. On the other hand, in winter the MCV and erythroblasts were inversely and proportionally correlated. For *M. micans*, micronuclei and nuclear abnormalities showed significant positive correlation. Erythroblasts were inversely correlated only with MCV.

Discussion

Prochilodus argenteus, popularly known as curimatã-pacu, is a fish endemic to the Basin of São Francisco river, economically important and appreciated by professional fishing. Moreover, is of great interest that the fish satisfactorily respond to reproductive technologies and market acceptance (SATO et al., 1996). The values of MCV in the present study were similar to those reported in *Prochilodus lineatus* by Ranzani-Paiva and Godinho (1985).

The values observed in Ht directly reflect the increased levels of erythropoiesis, since the Er and MCV had high values during summer. Val et al. (1992), determined in *Prochilodus nigrans* of Amazonian river, values lower than those registered in our study. In this way, although the fish belong to the same family, we can observe that there is an interspecific variation and influence of latitude, seasonality and physical and chemical parameters of water.

The average of erythrocytes in our study was similar to those observed by Ranzani-Paiva et al. (2000) in *P. lineatus* from the Paraná river. The highest values of these cells in the summer may be directly related to the increase in metabolism, due to the increase in water temperature, which stimulates erythropoiesis in an attempt to increase tissue oxygenation as observed by Lecklin and Nikinmaa (1998).

The reduction in the percentage of lymphocytes in the winter has also been observed by Dexiang and Ainsworth (1991). Possibly this may be compensated by increased phagocytic capacity. According to Ellis (1981) this lymphopenia is influenced by climatic conditions, especially in the colder seasons (winter and autumn), when the production of adrenocorticotropic hormone (ACTH) and cortisol are altered. Moreover, the temperature may influence the mitogenic activity of the cells.

In *P. maculatus* we did not find any statistical difference in the erythrocyte indices, the absolute number of leukocytes and thrombocytes, which show low influence of seasonal factors on the hematological condition of the species. Compared with other species of the same family (Pimelodidae), as *Pseudoplastystoma corruscans*, Ranzani-Paiva et al. (2000) reported low values of Hb and Ht. Since then, we may observe that not always a phylogenetic connection, share certain physiological characteristics. For leukocytes, the neutrophils in *P. maculatus* were abundant in the summer, and similar to the found by Ranzani-Paiva and Eiras (1992).

The total number of leukocytes observed in *M. micans* was high when compared with other species of this family, as *P. mesopotamicus*, *C. macropomun* (TAVARES-DIAS et al., 1999a) and *Brycon amazonicus* (TAVARES-DIAS et al., 1999b). The total number of thrombocytes in the present study was lower than found by Tavares-Dias and Mataqueiro (2004) for *P. mesopotamicus*. For the immature cells, the value was lower than found by Tavares-Dias and Mataqueiro (2004) in *P. mesopotamicus*. The values found in this study are inedited for *M. micans* and can serve as basis for further studies

The thrombocytes, although the phagocytic issue is discussed in teleosts, this activity had been found in cartilaginous fish by Walsh and Luer (1998). Suzuki (1986) observed pseudopods in thrombocytes of *Oncorhynchus mykiss* indicating phagocytosis. Besides that, Veiga et al. (2000), in cytochemical studies, observed the presence of glycogen in the cytoplasm of *Salminus maxillosus*, and Nakaghi et al. (1995) observed the presence of peroxidase in *P. mesopotamicus*. In this context, we suggested a dual function for the thrombocytes, phagocytic and aggregatory, helping to control homeostasis (STOSKOPF, 1993; MATUSHIMA; MARIANO, 1996; MARTINS et al., 2006).

In the present study the variation in erythrocyte indices does not allow attributing to pollution, but the increase in MCV is indicative of stress including that caused by pollutants. In this way, some of the high values comparing to other studies may, in principle, be attributed to seasonality.

Correlation between micronuclei and nuclear abnormalities show that each morphological change may be the manifestation from micronucleus in erythrocyte of *M. micans*. In the other species we not found any correlation between the frequency of micronuclei, nuclear abnormalities, erythroblasts and erythrocytes. Al-Sabti and Metcalfe (1995) report that, under experimental conditions, the frequency of micronuclei in erythrocytes is highly dependent on the levels of hematopoiesis. However, there was no correlation between the increase of erythrocytes and erythroblasts with micronuclei.

In relation to nuclear abnormalities in erythrocytes, there are few explanations for that, in fact, its origin is confirmed. Among the most accepted, we highlight the study by Shimizu et al. (1998). These authors demonstrated that the cell to detect an affected region began a process of repair and elimination of chromatin. The affected part is then moved to the periphery of the nucleus and eliminated by exocytosis. Thus, prior the complete elimination, the nuclear membrane may present imperfections, characterizing the nuclear abnormalities.

The finding of higher number of nuclear abnormalities in micronuclei indicates that the mechanism of exocytosis can be interrupted; not being very efficient, because frequently it cannot eliminate completely the fragment inside the nucleus, remaining in the periphery of nuclear membrane. Other indicium may be the evidence of oxidative stress induced by an environmental tensor. Thus, in oxidative stress, one of the first targets is the membranes that, by having its permeability and selectivity altered by lipid peroxidation, becoming the nucleus more susceptible and, therefore, might to form nuclear abnormalities and micronuclei, respectively.

Conclusion

From the results obtained, it is expected that eventually the hematological values may contribute to assess the effects of pollutants and can be used to monitor environmental quality in rivers and captivity.

The frequency of micronuclei and nuclear abnormalities found was not significantly different between sampling periods and between rivers São Francisco and Paracatu, however, the highest values were found in summer. This possibly can be attributed to increased metabolism and consequent formation of endogenous metabolites that can make the membranes more permeable and susceptible to physical and chemical agents. Furthermore, we observed a positive correlation between the increase of micronuclei and nuclear abnormalities in *M. micans.* This data about frequency of micronuclei, nuclear abnormalities and erythroblasts are unprecedented for these species and may serve as basic data for the adoption of these biomarkers in studies of environmental pollution.

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